PB81-213209

Effects of Post-Implantation Exposure to Selected Pesticides on Reproductivity in Rats

Southern Univ.
Baton Rouge, LA

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Jun 81

U.S. Department of Commerce
National Technical Information Service
NTIS

PB81-213209

EPA 600/1-81-048
June 1981

EFFECTS OF POST-IMPLANTATION EXPOSURE TO SELECTED PESTICIDES ON REPRODUCTIVITY IN RATS

by

Fitzgerald Spencer
Department of Biological Sciences and
Health Research
Southern University
Baton Rouge, LA 70813

Grant No. R804974

Project Officer

August Curley

Environmental Toxicology Division
Health Effects Research Laboratory
Research Triangle Park, NC 27711

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

PRODUCT OF:
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/1-81-048	2. ORD Report
3. RECIPIENT'S ACCESSION NO. PB81 213209	
4. TITLE AND SUBTITLE: EFFECTS OF POST-IMPLANTATION EXPOSURE TO SELECTED PESTICIDES ON REPRODUCTIVITY IN RATS	5. REPORT DATE: June 1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): Fitzgerald Spencer	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Department of Biological Sciences and Health Research, Southern University, Baton Rouge, LA 70813	10. PROGRAM ELEMENT NO. CCAE1A
	11. CONTRACT/GRANT NO. R804974
12. SPONSORING AGENCY NAME AND ADDRESS: Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 RTP, NC	13. TYPE OF REPORT AND PERIOD COVERED: Final
	14. SPONSORING AGENCY CODE: EPA 600/11
15. SUPPLEMENTARY NOTES: Project Officer: August Curley	

16. ABSTRACT

The post-implantational effects of dinoseb, PCBs (Aroclor 1254), rotenone and zineb on reproductive systems were examined using decidualized pseudopregnant rat as a model. Uterine protein, uterine glycogen, uterine water, and ovarian protein were studied in day 10 decidualized pseudopregnant rats fed the toxicants from days 6 through 9 of pseudopregnancy. Dinoseb reduced uterine protein and uterine glycogen in rats fed 25 ppm and higher concentrations. Uterine water and uterine weight were reduced at the highest dosage of 750 ppm. Ovarian protein was diminished at 150 ppm and higher concentrations. PCBs lowered uterine glycogen, but uterine protein content was not reduced in a dose-related manner. Ovarian protein content was diminished at 50 ppm and higher concentrations. Uterine weight and uterine water were not changed in rats fed up to 1000 ppm of the PCBs. Rotenone reduced uterine protein in rats fed 200 ppm and higher concentrations. In day-16 pregnant rats fed rotenone (100, 200, 400, and 600 ppms) from days 6-;6 of pregnancy, placental protein, placental glycogen, ovarian protein, and maternal body weight were reduced. Additionally, these dosing regimens reduced fetal survival rate. Fetal weight of fetus delicered from rotenone-fed dams was not affected. The decidualized pseudopregnant uterine, and placental functions, and fetal survival rate of rats were not affected by zineb up to 2500 ppm.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)*: UNCLASSIFIED	21. NO. OF PAGES: 33
	20. SECURITY CLASS *(This page)*: UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (9-73)

DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U. S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U. S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Few investigations have examined the effects of pesticides on mammalian reproductive systems although the presence of many classes of pesticides in our environment leave no doubt on the necessity of testing possible teratogenic and fetotoxic effects of compounds that may be encountered by pregnant women. This study addresses this area.

F. G. Hueter, Ph.D.
Director,
Health Effects Research Laboratory

ABSTRACT

The post-implantational effects of dinoseb, PCBs (Aroclor 1254), rotenone and zineb on reproductive systems were examined using decidualized pseudopregnant rat as a model. Uterine protein, uterine glycogen, uterine water, and ovarian protein were studied in day-10 decidualized pseudopregnant rats fed the toxicants from days 6 through 9 of pseudopregnancy. Dinoseb reduced uterine protein and uterine glycogen in rats fed 25 ppm and higher concentrations. Uterine water and uterine weight were reduced at the highest dosage of 750 ppm. Ovarian protein was diminished at 150 ppm and higher concentrations. PCBs lowered uterine glycogen, but uterine protein content was not reduced in a dose-related manner. Ovarian protein content was diminished at 50 ppm and higher concentrations. Uterine weight and uterine water were not changed in rats fed up to 1000 ppm of the PCBs. Rotenone reduced uterine protein in rats fed 200 ppm and higher concentrations. Uterine glycogen was diminished at 10 ppm and higher concentrations. In day-16 pregnant rats fed rotenone (100, 200, 400, and 600 ppms) from days 6-15 of pregnancy, placental protein, placental glycogen, ovarian protein, and maternal body weight were reduced. Additionally, these dosing regimens reduced fetal survival rate. Fetal weight of fetus delivered from rotenone-fed dams was not affected. The decidualized pseudopregnant uterine, and placental functions, and fetal survival rate of rats were not affected by zineb up to 2500 ppm.

CONTENTS

FOREWORD..... iii
ABSTRACT..... iv
LIST OF ILLUSTRATIONS..... vi
LIST OF TABLES..... vii
INTRODUCTION..... 1
MATERIALS AND METHODS..... 2
METHODS..... 2
Animals and care..... 2
Decidualized pseudopregnancy..... 2
Pregnancy studies..... 2
Biochemical studies..... 3
Feeding..... 3
Protein determination..... 3
Glycogen determination..... 4
Water content determination..... 4
Statistic analysis..... 4
MATERIALS..... 4
RESULTS AND DISCUSSION..... 5
CONCLUSIONS..... 21
RECOMMENDATION..... 22
REFERENCES..... 23

LIST OF ILLUSTRATION

Number		Page
1	Dinoseb on uterus	7
	a. Uterine weight	
	b. Water content	
	c. Protein content	
	d. Glycogen content	
2	a. Dinoseb on decidualized pseudopregnant rats	8
	b. Dinoseb on pregnant rats	8
3	PCB on DCR PSPG rats	9
4	PCB on uterus	9
	a. Protein content	
	b. Glycogen content	

LIST OF TABLES

Number		Page
1	Uterine weight and uterine water content of Day 10 decidualized pseudopregnant rats given PCB (Aroclor 1254)	10
2	Uterine weight and uterine water content of Day 10 decidualized pseudopregnant rats given rotenone	11
3	Ovarian protein content of Day 10 decidualized pseudopregnant rats given rotenone	12
4	Uterine protein and uterine glycogen contents of Day 10 decidualized pseudopregnant rats given rotenone	12
5	Placental protein and placental glycogen contents of Day 16 pregnant rats given rotenone	13
6	Ovarian protein content of Day 16 pregnant rats given rotenone	14
7	The effect of rotenone on body weight of rats	15
8	The effect of rotenone on the outcome of pregnancy	16
9	Uterine weight, water, protein, and glycogen contents of Day 10 decidualized pseudopregnant rats given zineb	17
10	Ovarian weight and ovarian protein content of Day 10 of decidualized pseudopregnant rats given zineb	18
11	Placental protein and glycogen contents on Day 16 of pregnant rats given zineb	19
12	The effect of zineb on the outcome of pregnancy	20

SECTION 1

INTRODUCTION

The thalidomide episode caused great public concern (Lenz et al., 1962) and left no doubt on the necessity of testing possible teratogenic and fetotoxic effects of compounds that may be encountered by pregnant women.

Since the presence of many classes of pesticides in the environment offers numerous opportunities for these toxicants to enter the physiological systems of various species of animals including man, there has been an increase interest in studying the teratogenic effects of certain pesticides on mammalian reproduction. A review on teratology is offered by Wilson (1972). Few investigations have examined the effects of pesticides on mammalian reproductive systems. This study was conducted with this idea in mind.

Pesticides such as DDT has been shown to reduce estradiol titer in blood of ringdoves, Streptopelia risoria (Peakall, 1970a). On the basis of experiments, the egg-shell thinning action of chlorinated hydrocarbons has been attributed to direct estrogenic action of DDT and its analogs (PCBs) (Bitman et al., 1968).

Because pseudopregnant and pregnant rats have identical mechanism for controlling the onset and loss of uterine sensitivity (Carlson and De Feo, 1965), the deciduomal response in decidualized pseudopregnant rats could be used to study possible reproductive disruption as caused by various pesticides in pregnant uterus.

SECTION 2

MATERIALS AND METHODS

METHODS

Animals and care Virgin albino rats (Sprague-Dawley strain) from Holtzman Co., Wisconsin, were caged individually in an environmentally controlled room (temperature: 20± 2°C; photoperiod: 14 h light and 10 h dark). The animals were given Purina Lab Chow and tap water freely. The advancement of estrus stage of the rats was determined daily by microscopic examination on vaginal lavage. Rats (200-300 g) which exhibited two consecutive 4-5 day estrus cycles were selected for these studies.

Decidualized pseudopregnancy studies Pseudopregnancy was induced by mechanically stimulating the uterine cervix of the rat during the proestrus and estrus stages (De Feo, 1966). Day 1 of pseudopregnancy was designated as the first day when leukocytes appeared predominantly in the vaginal smear. The decidual cell reaction,which involved the proliferation of uterine deciduoma or maternal placental component, was induced by bilateral traumatization (Zarrow et al., 1964) at Day 4 of pseudopregnancy. On Day 10 of pseudopregnancy, animals were sacrificed. Ovaries were removed for protein determination. Glycogen, protein and water content determinations were conducted on the uterine horns.

Pregnancy studies Pregnancy was attained by housing a female rat in the estrus stage with a fertile male. The day on which sperm appeared in the vaginal smear was designated

as Day 1 of gestation. The number of implantation sites was counted on Day 6 of pregnancy, following a tail vein injection of Chicago blue dye (Labhsetwar, 1971). The number of developing embryos was counted on Day 12 of pregnancy during laparotomy. The percentage of resorption in implantation sites (I.S.) was calculated by the formula:

$$\frac{\text{Number of I.S. at Day 6 - Number of I.S. at Day 12}}{\text{Number of I.S. at Day 6}}$$

On the day of parturition, the number of dead and alive fetuses was recorded. The fetal survival index of the fetuses was calculated by the formula given by Pepe and Rothchild (1973). The litters were weighed at 0-36 h upon delivery.

Biochemical studies Pregnant rats were sacrificed on Day 16 of pregnancy by cervical dislocation under light ether anesthesia. The ovaries from both uterine horns were extracted over ice for protein assay. The placenta from a living fetus was used for protein and glycogen determinations.

Feeding All toxicants were mixed in a pulverized lab chow to form a desired dietary concentration. Rats were given free access to the diet during the test. Food consumption, and body weights were monitored for each individual. Feeding was conducted from days 6-15 of pregnancy and days 6-9 of decidualized pseudopregnancy. These feeding periods coincided with the period of elevated plasma titers of progesterone during pregnancy (Pepe and Rothchild, 1974) and the period of deciduomal growth during pseudopregnancy (Zarrow et al., 1964).

Protein determination Protein analysis was based on the method Lowry et al. (1951) using bovine serum albumin as the standard. Fresh tissue sample was grinded in a

chilled Potter Elvehjem homogenizer with glass distilled water to form a 2.0% homogenate (g/ml). Following an incubation period of 15 minutes at 37°C, the homogenate was centrifuged at 15,000xg at 2°C for 20 minutes. Absorbance values were read on a Beckman (Model 24) spectrophotometer at 660mu.

Glycogen determination Glycogen analysis was based on the method of Seifter et al. (1950), modified by Zarrow et al. (1964), using Type III rabbit liver glycogen as the standard. Absorbance values were read on a Bausch and Lomb Spectronic 20 colorimeter set at 620 mu.

Water content determination For water content determination, which is the difference between the wet and dry weights, one uterine horn was dried in an oven at 80-100°C for 48 h.

Statistic analysis Analysis was accomplished by one-way analysis of variance and the Student's t-test. Differences among the experimental groups were tested for statistical significance using Duncan multiple range test (Duncan, 1955). Mann-Whitney U test was applied wherever the collected data were deemed as appropriate.

MATERIALS

Dinoseb, PCB (Aroclor 1254), rotenone and zineb were supplied by the Environmental Protection Agency, Research Triangle Park, North Carolina. Stock diets of the toxicants were prepared as in the following tabulated scheme:

Compound	Quantity	Base ingredients	Quantity	Vehicle
Dinoseb	1 g	Crushed chow	99 g	Acetone
PCB	1 ml	Acetone	99 ml	--
Rotenone	1 g	Crushed chow	99 g	No
Zineb	1 g	Crushed chow	99 g	No

From the stock diet, a calculated amount was then drawn off, and further mixed with an additional amount of pulverized chow to form a desired dietary concentration in the rat chow.

SECTION 3

RESULTS AND DISCUSSION

As shown in Fig. 1a and Fig. 1b, uterine weight and uterine water content of decidualized pseudopregnant rats exposed to dinoseb were only reduced at the highest dosage of 750 ppm. In comparison with the control, uterine protein and uterine glycogen contents were reduced at 25 ppm and higher concentrations (Fig. 1c and Fig. 1d). Ovarian protein content of the rats exposed to dinoseb for 4 days was not affected up to 500 ppm (Fig. 2a). The difference in susceptibility to dinoseb, between uterine protein content (Fig. 1c) and ovarian protein content (Fig. 2a) might be attributed to a differential distribution of dinoseb within the reproductive system (Wayland, 1975). In pregnant rats, exposed to dinoseb for 10 days, ovarian protein content was reduced at 200 ppm and higher concentrations (Fig. 2b).

Ovarian protein of the decidualized pseudopregnant (DCR PSPG) rats exposed to the PCB, Aroclor 1254, was reduced at 50 ppm and higher concentrations (Fig. 3). Uterine protein content was reduced at 25 ppm and higher concentrations (Fig. 4a). This reduction was not dose-dependent, and the difference was ascribed to abnormal control value (Weil and Carpenter, 1969). Uterine glycogen content of the rats was reduced at 150 ppm and higher concentrations (Fig. 4b). Uterine weight and uterine water content regardless of the dose of the

PCBs under test were not affected (Table 1).

In DCR PSPG rats exposed to rotenone, uterine weight was reduced at 100 ppm and higher concentrations, but uterine water content was not affected (Table 2). Ovarian protein content was not reduced up to 750 ppm (Table 3). Uterine protein content was reduced at 200 ppm, in comparison with the control (Table 4). Uterine glycogen content was decreased at 10 ppm and higher concentrations. Placental protein content and placental glycogen content of pregnant rats exposed to rotenone were reduced (Table 5). Ovarian protein content of the rats was diminished (Table 6). At high level of exposure to rotenone, maternal body weight of the rats was decreased (Table 7). As shown in Table 8, rotenone did not affect the developing embryos. Fetal survival rate of the fetuses was decreased at 100 ppm and higher concentrations. Fetuses delivered from dams exposed to rotenone during the post-implantational stage of pregnancy did not exhibit any reduction in body weight.

In Table 9, at the levels of zineb tested, intrauterine environment,in terms of uterine protein, uterine glycogen, and uterine water contents, of the rats was not affected. Ovarian protein in both the pseudopregnant and pregnant rats exposed to zineb for 4 days and 10 days,respectively, were not affected (Table 10). Placental protein was not affected by zineb (Table 11). In comparison with the control, placental glycogen content was increased. This increase was not dose-dependent. In Table 12, development of implantation sites in pregnant rats after 7 days of exposure to zineb was not affected. The fetal survival rate was not affected. Fetal weight was reduced but this decrease was not regarded to be compound-related since it was not dose dependent.

Fig 1a

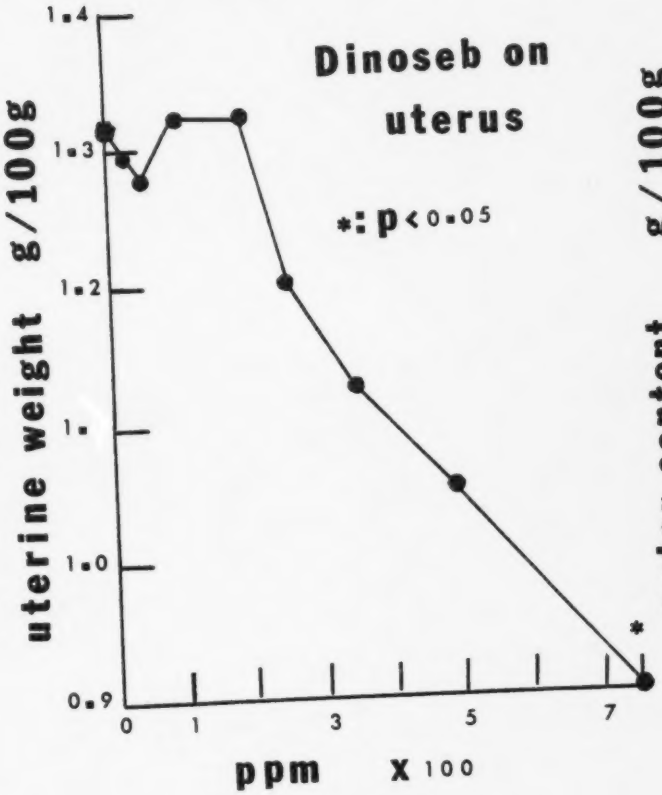

Fig 1b

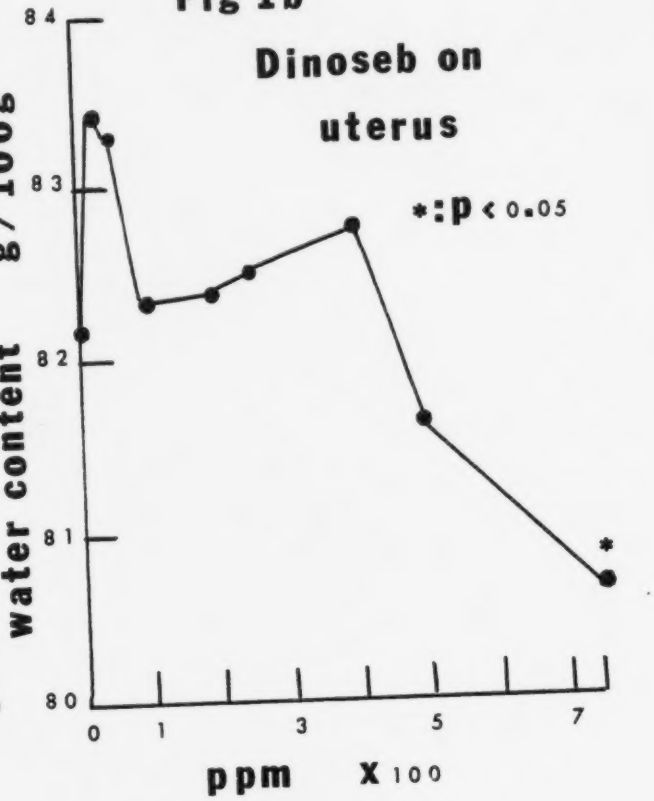

Fig 1c

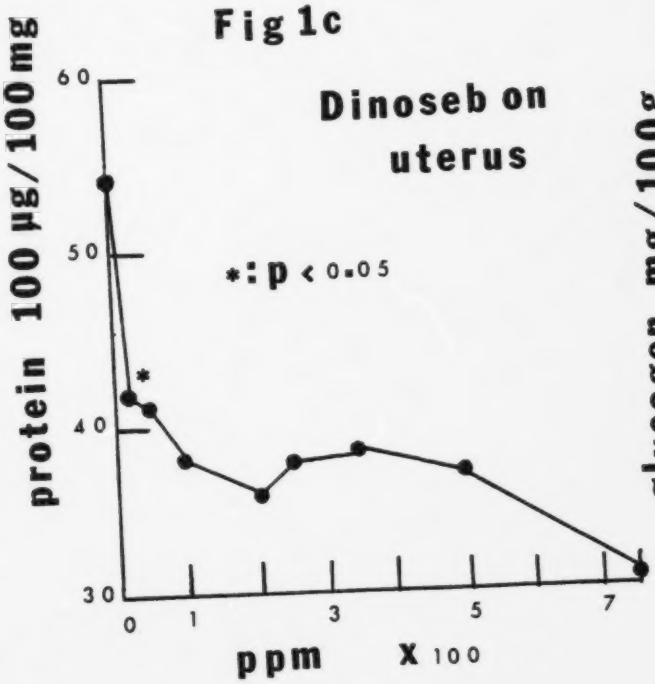

Fig 1d

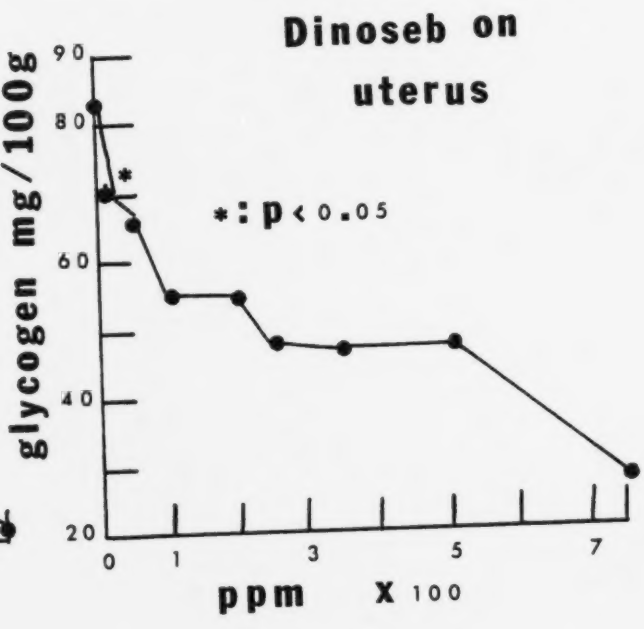

Fig 2a

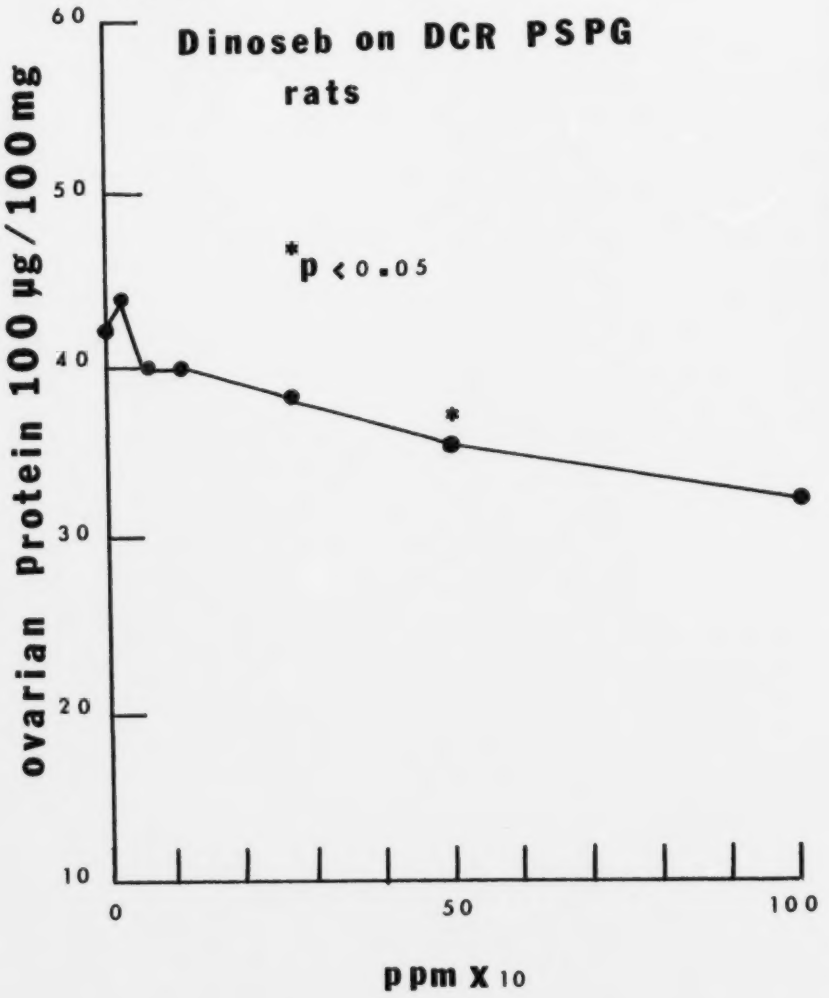
Dinoseb on DCR PSPG rats
*p < 0.05
ovarian protein 100 µg/100 mg
60
50
40
30
20
10
0
50
100
ppm X 10

Fig 2b

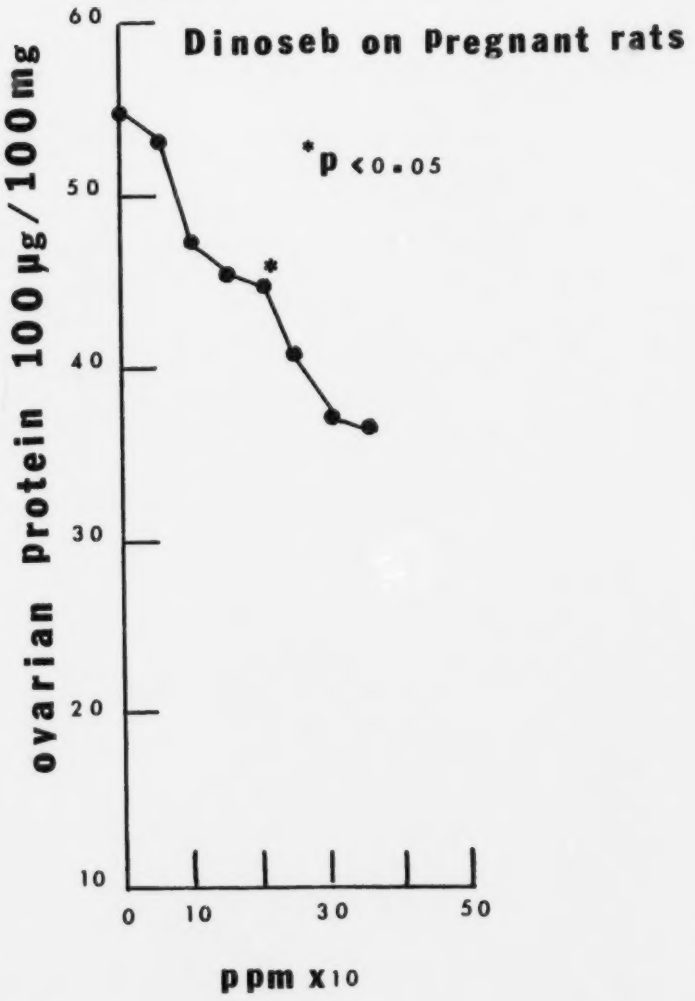
Dinoseb on Pregnant rats
*p < 0.05
ovarian protein 100 µg/100 mg
60
50
40
30
20
10
0
10
30
50
ppm X 10

Fig 3

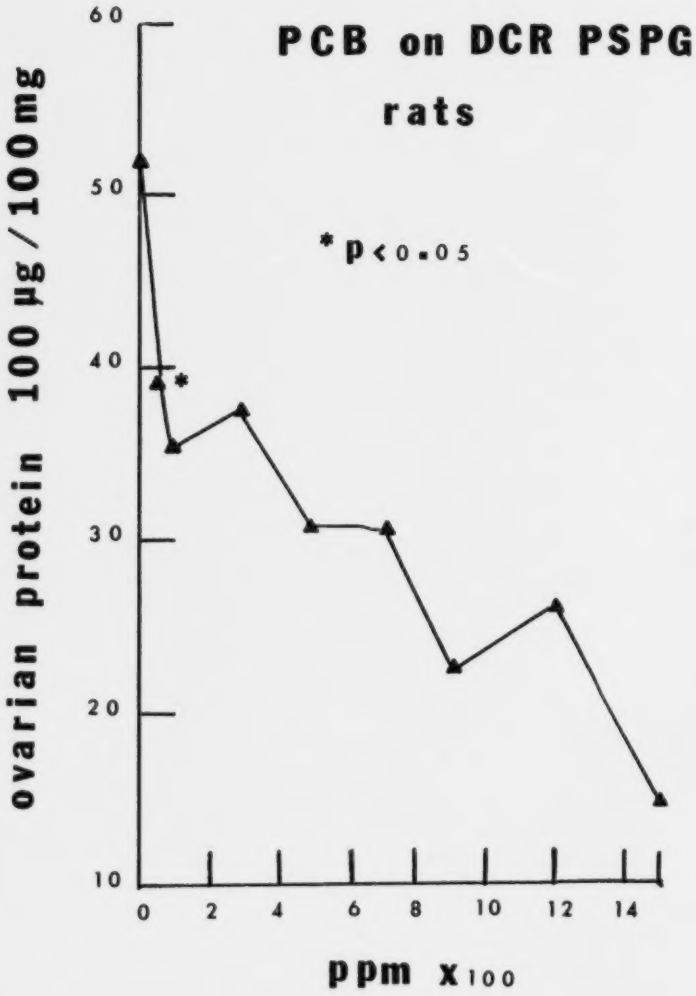

Fig4a

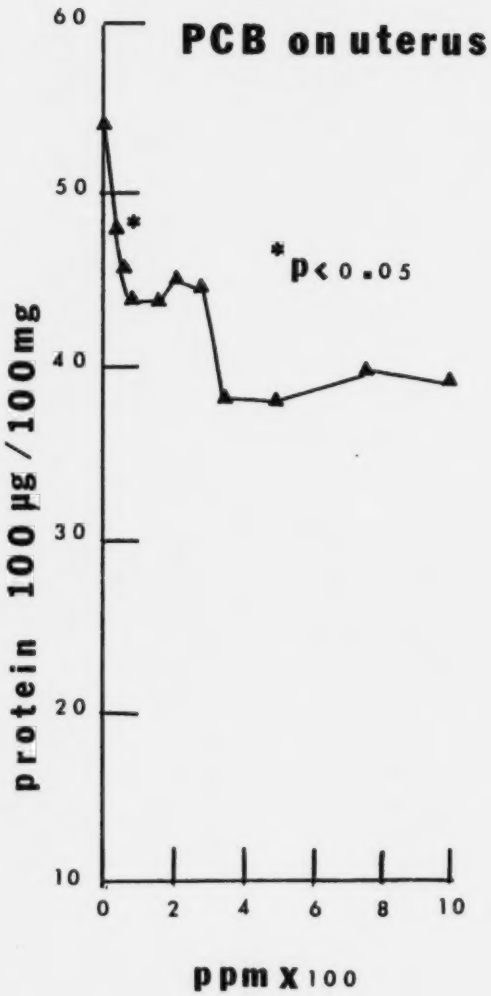

Fig4b

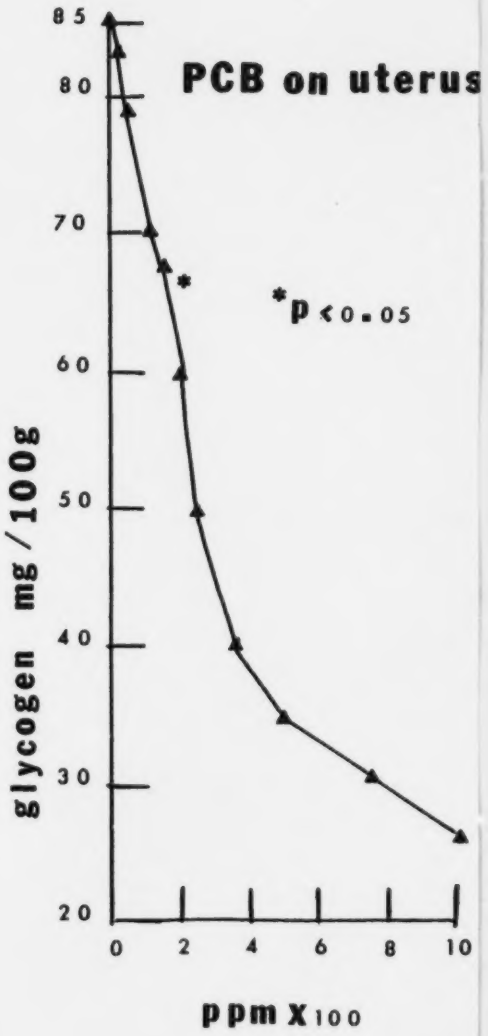

TABLE 1

UTERINE WEIGHT AND UTERINE WATER CONTENT OF DAY 10 DECIDUALIZED PSEUDOPREGNANT RATS GIVEN PCB (AROCLOR 1254).

Diet[a] PPM	Daily intake of PCB (mg/day) $\bar{x}\pm SE$	Uterine weight (g) $\bar{x}\pm SE$	Uterine water content[b] (g/100g) $\bar{x}\pm SE$
control	-----	3.18±0.24	82.2±0.5
25	0.96±0.04	2.90±0.18	82.5±0.3
50	1.87±0.03	2.68±0.27	82.9±0.2
100	3.84±0.05	2.76±0.26	82.8±0.3
150	4.97±0.11	2.93±0.29	83.2±0.4
200	7.47±0.21	3.17±0.20	83.6±0.6
250	8.82±0.27	3.13±0.11	83.3±0.2
350	13.00±0.40	3.30±0.37	82.5±0.2
500	15.69±0.28	2.50±0.22	82.5±0.3
750	21.42±0.95	2.41±0.17	82.3±0.4
1000	23.52±1.36	2.38±0.08	82.5±0.5
ANOVA	-----	NS	NS

[a] Rats were given a diet of respective concentrations from days 6 through 9 of decidualized pseudopregnancy.

[b] Percentage= $\frac{\text{wet weight (g) - dry weight (g)}}{\text{wet weight (g)}} \times 100$

TABLE 2

UTERINE WEIGHT AND UTERINE WATER CONTENT OF DAY 10 DECIDUALIZED PSEUDOPREGNANT RATS GIVEN ROTENONE

Diet[a] PPM	N	Daily chow intake (g/day) $\bar{x}\pm SE$	Daily intake of rotenone (mg/kg/day) $\bar{x}\pm SE$	Body weight[b] of rats (g) $\bar{x}\pm SE$	Uterine weight (g/100g) $\bar{x}\pm SE$	Uterine water content (g/100g) $\bar{x}\pm SE$
control	10	17.0±1.2	-----	257± 6	1.46±.04	82.8±0.3
10	10	18.5±0.5	0.74±0.02	248± 4	1.39±.01	83.2±0.4
100	10	18.4±0.5	7.08±0.17	262± 3	1.24±.10[c]	82.6±0.5
200	10	17.6±0.6	14.08±0.45	248± 6	1.26±.09[c]	82.4±0.5
250	10	15.9±0.7	15.90±0.70	249± 6	1.05±.10[c]	82.2±0.8
500	10	12.5±1.1	26.03±2.33	239±10	1.13±.11[c]	82.0±0.3
750	10	10.5±0.8	32.82±2.60	240± 6	1.10±.10[c]	81.7±0.6
1000	10	9.0±0.6	40.91±1.49	218±12	1.03±.06[c]	81.5±1.2
ANOVA		-----	-----	$p<0.05$	$p<0.05$	NS

[a]Rats were given a diet of respective concentrations from days 6 through 9 of decidualized pseudopregnancy.

[b]Body weight of rats was measured on day 9 of pseudopregnancy.

[c]$p<0.05$ in comparison with the control, using Student's t-test.

TABLE 3

OVARIAN PROTEIN CONTENT OF DAY 10 DECIDUALIZED PSEUDOPREGNANT RATS GIVEN ROTENONE

Diet[a] PPM	N	Ovarian weight (g) $\bar{x}\pm SE$	Ovarian protein content (100µg/100mg tissue) $\bar{x}\pm SE$	
control	5	0.10±.01	29.6±7.4	b
100	5	0.10±.00	29.4±4.2	
250	5	0.10±.00	23.2±5.7	
500	5	0.08±.00	27.4±9.4	
750	5	0.08±.00	19.5±8.7	
1000	5	0.08±.00	17.4±1.7	
ANOVA		$p < 0.05$	$p < 0.05$	

[a]Rats were given a diet of respective concentrations from days 6 through 9 of decidualized pseudopregnancy.

[b]Values joined by the same line represent cohorts of values which do not differ from each other.

TABLE 4

UTERINE PROTEIN AND UTERINE GLYCOGEN CONTENTS OF DAY 10 DECIDUALIZED PSEUDOPREGNANT RATS GIVEN ROTENONE

Diet[a] PPM	Uterine protein content[b] (100µg/100mg) $\bar{x}\pm SE$		Uterine glycogen content[b] (mg/100g tissue) $\bar{x}\pm SE$	
control	53.4±3.4	c	84.98±6.50	c
10	50.3±3.3		72.70±4.58	
100	46.9±2.5		57.11±4.71	
200	45.6±4.4		59.30±4.06	
250	41.0±2.7		54.94±3.14	
500	44.1±3.8		57.81±6.07	
750	40.3±1.2		50.02±5.57	
1000	36.9±3.7		46.10±6.44	
ANOVA	$p < 0.05$		$p < 0.05$	

[a]Rats were fed with a diet of respective concentrations from days 6 through 9 of decidual ed pseudopregnancy.

[b]N=10 for all cases.

[c]Values joined by the same line represent cohorts of values which do not differ from each other.

TABLE 5

PLACENTAL PROTEIN AND PLACENTAL GLYCOGEN CONTENTS OF DAY 16 PREGNANT RATS GIVEN ROTENONE

Diet[a] PPM	N	Maternal body weight (g) $\bar{x}\pm$SE	Placental protein (100µg/100mg) $\bar{x}\pm$SE		t-test (p)	Placental glycogen (mg/100g) $\bar{x}\pm$SE		t-test (p)
control	7	321± 9	54.4±4.6	b	-----	482±23	b	-----
100	7	200±11	57.1±2.6		NS	495±19		NS
200	7	204±13	41.6±6.1		<0.05	462±62		NS
400	7	175±14	39.6±7.3		<0.05	351±29		<0.05
600	7	171± 8	38.0±2.1		<0.05	343±15		<0.05
ANOVA		-----	p<0.05			p<0.05		

[a]Rats were fed with a diet of respective concentrations from Days 6 through 15 of pregnancy.

[b]Values joined by the same line represent cohorts of values which do not differ from each other.

TABLE 6

OVARIAN PROTEIN OF DAY 16 PREGNANT RATS GIVEN ROTENONE

Diet[a] PPM	Daily chow intake[b] (g/day) $\bar{x}\pm$SE	Daily intake of rotenone[b] (mg/kg/day) $\bar{x}\pm$SE	Ovarian protein content[b] (100µg/100mg) $\bar{x}\pm$SE	t-test (p)
control	19.7±1.3	-----	47.9±3.0 [c]	---
100	19.6±1.5	9.8±0.8	46.0±3.4	NS
200	17.1±0.3	16.7±0.3	36.4±6.2	<0.05
400	11.1±0.7	25.3±1.7	33.9±2.5	<0.05
600	8.1±0.7	28.5±2.3	29.4±2.2	<0.05
800	5.7±0.3	28.1±1.6	25.0±1.1	<0.05
ANOVA	-----	-----	p<0.05	

[a]Rats were given a diet of respective concentrations from days 6 through 15 of pregnancy.

[b]N=7 in all cases.

[c]Values joined by the same line represent cohorts of values which do not differ from each other.

TABLE 7

THE EFFECT OF ROTENONE ON BODY WEIGHT OF PREGNANT RATS

Diet[a] PPM	N	Daily chow intake (g/day) $\bar{x}$±SE	Daily intake of rotenone (mg/kg/day) $\bar{x}$±SE	Maternal body weight at Day 6 (g) $\bar{x}$±SE	Maternal body weight at Day 12 (g) $\bar{x}$±SE	Maternal body weight at Day 16 (g) $\bar{x}$±SE
control	7	-----	-----	232± 8	247±12	265±12
10	7	19.8±0.9	0.77±.03	221± 6	236± 7	258± 6
100	7	19.2±0.5	8.10±.02	226± 6	228± 7	244± 9
200	7	17.3±0.3	12.80±.23	254± 7	259± 9	272± 8
400	7	8.5±0.5	16.50±.95	239±11	221±11	205±10
600	7	6.2±0.3	19.20±.81	230±15	206±12	196±14
800	7	5.4±0.2	22.80±.89	262±12	226± 9	195±11
ANOVA		----	----	-----	$p < 0.05$	$p < 0.05$

[a]Rats were fed with a diet of respective concentrations from days 6 through 15 of pregnancy.

TABLE 8

THE EFFECT OF ROTENONE ON THE OUTCOME OF PREGNANCY IN RATS

Diet[a] PPM	Number of litter	Implantations at Day 6 per dam $\bar{x}$±SE	Number of conceptuses at Day 12 $\bar{x}$±SE	Percentage of fetal survival rate per litter $\bar{x}$±SE	t-test (p)	Average fetal weight per litter (g) $\bar{x}$±SE
control	7	12.3±1.2	12.3±1.2	67.5± 8.1	-----	6.94±0.13
10	7	12.3±0.4	12.3±0.4	60.8± 8.8	NS	7.53±0.32
100	7	12.6±0.5	12.6±0.5	32.7±11.5	< .05	6.66±0.22
200	7	11.0±0.7	11.0±0.7	23.5± 7.2	< .05	7.00±0.12
400	7	12.6±0.5	12.6±0.5	23.3±11.5	< .05	7.02±0.34
600	7	11.0±0.6	11.0±0.6	22.4±12.6	< .05	6.49±0.41
800	7	11.4±0.6	11.4±0.6	15.8± 4.6	< .05	7.05±0.15
				$p<0.05$[b]		NS[c]

[a]Rats were given a diet of respective concentrations from days 6 through 15 of pregnancy

[b]Effects of treatment vs. control, using analysis of variance.

[c]Effects of treatment vs. control, using Mann-Whitney U test.

TABLE 9

UTERINE WEIGHT, WATER, PROTEIN AND GLYCOGEN CONTENTS OF DAY 10 DECIDUALIZED PSEUDOPREGNANT RATS GIVEN ZINEB

Diet[a] (PPM)	Daily intake[b] of zineb (mg/day)	Body weight[b] of rats (g)	Uterine[b] weight (g)	Uterine[b,c] water content (%)	Uterine[b] protein (100µg/100mg uterus)	Uterine[b] glycogen (mg/100g uterus)
control	-----	241±11	3.8±0.3	81.0±0.7	45.6±3.6	111±21
500	10.1±0.4	258± 9	3.2±0.2	80.3±0.6	41.4±4.4	89±17
1000	21.6±0.8	271± 7	3.4±0.4	80.7±0.8	37.7±5.1	109±39
1500	31.1±0.6	249±11	3.6±0.3	79.8±0.3	39.6±4.5	100±28
2000	42.9±1.1	270± 4	3.2±0.4	79.6±0.5	34.0±2.4	99±10
	$p < 0.05$[d]	NS[d]	NS[d]	NS[d]	NS[d]	NS[d]

[a] Rats were given a diet of respective concentrations from Days 6 through 9 of decidualized pseudopregnancy.

[b] Mean±SE, for results of 5 rats.

[c] Percentage (%)= $\frac{\text{wet weight (g)} - \text{dry weight (g)}}{\text{wet weight (g)}} \times 100$

[d] Probability of treatment effect (control vs exposed groups), using analysis of variance.

TABLE 10

OVARIAN WEIGHT AND OVARIAN PROTEIN CONTENT ON DAY 10 OF DECIDUALIZED PSEUDOPREGNANT RATS AND ON DAY 16 OF PREGNANT RATS GIVEN ZINEB

Diet[a]	Pseudopregnant rats		Pregnant rats	
(PPM)	Ovarian[b] weight (g)	Ovarian[b] protein (100µg/100mg uterus)	Ovarian[b] weight (g)	Ovarian[b] protein (100µg/100mg uterus)
control	0.07±.01 [c]	50.3±8.0	0.16±.02	39.1±5.1 [c]
500	0.11±.00	48.4±2.6	0.16±.01	40.5±3.7
1000	0.10±.00	51.1±5.3	0.11±.02	34.6±4.0
1500	0.09±.01	52.6±8.8	0.15±.01	39.3±5.6
2000	0.10±.00	50.6±5.7	0.15±.02	36.4±4.5
2500	0.10±.00	47.1±7.8	0.13±.00	55.3±1.9
	$p < 0.05$[d]	NS[d]	NS[d]	$p < 0.05$[d]

[a] Rats were given a diet of respective concentrations from Days 6 through 9 of decidualized pseudopregnancy, or from Days 6 through 15 of pregnancy.

[b] Mean±SE, for results of 5 decidualized pseudopregnant rats and for results of 6 pregnant rats.

[c] Values joined by the line represent a cohort of values which do not differ significantly from each other.

[d] Probability of treatment effect (control vs exposed groups), using analysis of variance.

TABLE 11

PLACENTAL PROTEIN AND GLYCOGEN CONTENTS ON DAY 16 OF PREGNANT RATS GIVEN ZINEB

Diet[a] (PPM)	Daily[b] intake of zineb (mg/day)	Maternal[b] body weight (g)	Placental[b] protein (100µg/100mg tissue)	Placental[b] glycogen (mg/100g tissue)	
control	---	297±16	52.2±4.9	479±22	
500	11.8±0.6	294± 9	49.7±3.5	588±27	c
1000	21.1±1.3	269±11	53.8±2.8	554±94	
1500	30.3±2.4	298± 8	59.1±3.0	528±81	
2000	38.6±4.9	285± 5	51.7±2.1	573±73	
2500	54.8±0.3	285± 2	56.3±1.6	654±12	
		NS[d]	NS[d]	$p < 0.05$[d]	

[a] Rats were given a diet of respective concentrations from Days 6 through 15 of pregnancy.

[b] Mean±SE, for results of 6 rats.

[c] Values connected with the same line represent a cohort of values which do not differ from each other.

[d] Probability of treatment effect (control vs. exposed groups), using analysis of variance.

TABLE 12

THE EFFECT OF ZINEB ON THE OUTCOME OF PREGNANCY

Diet[a]	Daily intake of zineb (mg/day) x̄±SE	Number of litter	Implantations at Day 6 per dam x̄±SE	Number of conceptuses at Day 12 x̄±SE	Number of fetuses at birth		Percentage of fetal survival rate per litter x̄±SE	Fetal weight (g) x̄ ± SE		Maternal[b] weight gain (g) x̄±SE	
					Dead	Alive					
control	-----	6	13±0.5	13±0.5	33	45	57.2± 8.8	7.2±0.3	[c]	70±6	
500	10.0±0.5	6	13±0.4	13±0.4	33	44	58.7±12.2	7.3±0.2		56±3	
1000	19.8±1.1	6	10±0.6	10±0.6	28	34	54.3± 6.8	7.2±0.3		53±3	c
1500	31.5±0.5	6	11±0.6	11±0.6	23	40	63.2±12.3	6.7±0.3		50±2	
2000	41.5±0.6	6	10±0.3	10±0.3	29	29	49.0±9.2	7.1±0.1		51±6	
2500	51.7±1.2	6	12±0.3	12±0.3	38	31	46.4±2.9	6.9±0.1		45±2	
							NS[d]	$p < 0.05$[d]		$p < 0.05$[d]	

[a] Rats were given a diet of respective concentrations from Days 6 through 15 of pregnancy.

[b] Maternal weight gain= maternal weight after delivery (g) - maternal weight at day 6 (g)

[c] Values joined by the same line represent a cohort of values which do not differ significantly from each other.

[d] Probability of treatment effect (control vs. exposed groups), using analysis of variance.

SECTION 4

CONCLUSIONS

(1). Dinoseb, PCBs (Aroclor 1254), and rotenone are uterotoxic, causing disruption on the integrity of the decidualized pseudopregnant uterus. Since the intrauterine environment is maintained by sex hormones, namely, estrogen and progesterone, anti-estrogenic, and anti-progestogenic properties of these toxicants could be inferred.

(2). Rotenone interferes with fetal development. Because development of the placenta corresponds with that of the fetus, any interference imposed on the placenta by rotenone may be viewed as essentially disruptive to the fetal developmental process. In addition, rotenone is fetotoxic. The fetotoxic potential of rotenone is designated by a decrease in fetal survival rate.

(3). This study shows that zineb has low toxic effects on the maternal reproductive system and the fetus. This could be attributed to the fact that zineb does not accumulate to a large extent in mammalian tissues and could be metabolized readily.

RECOMMENDATION

1. Further studies on the toxicological effects of pesticides upon the reproductive system should be correlated with the adrenal functions. Results from past studies have shown that ovarian functions were affected by the adrenals in normal cyclic rats (1) and in pregnant rats (2). Additionally, the growth of decidualized pseudopregnant uterus was influenced by progesterone secreted by the adrenals (3).

2. Consideration should be given to dietary intake of the animal during any toxicological studies in reproduction. Starvation has been shown to be a contributing factor in the reduction of fetal and placental functions (4).

Footnotes

(1). Bartosik, D., Szarowski, D.H., and Watson, D.J. Endocrinology (88): 1425-1428, 1976.
(2). Poulton, B.R. and Reece, R.P. Endocrinology (61): 217, 1957.
(3). Manning, J.P. J. Endocrinol. (50): 451-455, 1971.
(4). Campbell, R.M., Innes, R.I. and Kosterlitz, H.W. J. Endocrinol. (9): 68-75, 1953.

References

1. Bitman, J., Cecil, H. C., Harris, S.J. and Fries, G.F. (1968). Estrogenic activity of o,p'-DDT in the mammalian uterus and avian oviduct. Science 168: 592-594.

2. Carlson, J. and De Feo, V.J. (1964). Quantitative studies on deciduoma formation and implantation in the lactating rat. Endocrinology 75 (6): 883-892.

3. De Feo, V.J. (1966). Vagical-cervical vibration: A simple and effective method for the induction of pseudopregnancy in the rat. Endocrinology 79:440.

4. Duncan, D.B. (1955). Multiple range and multiple F test. Biometrics 11: 1-42.

5. Labhsetwar, D.L. (1971). Progesterone F_2 alpha and implantation process in the rat. J. of Endocrinol. (5): 353-358.

6. Lenz, W., Pfeiffer, R.A., Koesnow, W. and Hayman, D.J. (1962). Thalidomide and congenital abnormalities. Lancet, 1: 45.

7. Lowry, O.H., Rosenberg, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with Folin phenol reagent. Biochem. 193: 265-272.

8. Peakall, D.B. (1970a). p,p'-DDT: Effects on calcium metabolism and concentrations of estradiol in the blood. Science 168: 592-594.

9. Peakall, D.B. (1970b). Pesticides and the reproduction in birds. Sci. Amer. 222: 72-78.

10. Pepe, G.J. and Rothchild, I. (1973). Serum progesterone levels in ovariectomized rats injected with progesterone and estrogen. Endocrinology 93: 1193-1198.

11. Seifter, S., Dayton, S., Novic, B. and Muntwyler, E. (1950). Glycogen estimation with anthrone reagent. Arch. in Biochem. 25: 191-200.

12. Weil, C.S. and Carpenter, C.P. (1969). Abnormal values in control groups during repeated-dose toxicologic studies. Toxicol. Appl. Pharmacol., 14: 335-339.

13. Wilson, J. G. (1972). Environmental effects on development-teratology. In: Pathophysiology of Gestation.

edited by N.S. Assali, Vol. 2, pp. 269-320. Academic Press, Inc., New York.

14. Zarrow, M.X., Yochim, J.M. and McCarthy, J.L. (1964). Experimental Endocrinology: A Sourcebook of Basic techniques, pp. 218-219. New York, London, Academic Press.